15/C

Appendix A; Response Mailed On 12/06/02
Unmarked Claims

1. (Restated) An enzyme-linked in-*situ* hybridization probe further characterized in that it comprises a probing nucleobase sequence that specifically hybridizes to a yeast specific target sequence.
2. (Restated) The probe of claim 1, wherein the target sequence is ribosomal RNA.
3. (Restated) The probe of claim 1, wherein the probe is a nucleic acid.
4. (Restated) The probe of claim 1, wherein the probe is a peptide nucleic acid.
5. (Restated) The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more species of yeast.
6. (Restated) The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more genus of a yeast.
7. (Restated) The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate all yeast in a sample.
8. (Restated) The probe of claim 1, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

C1

10. (Amended) An enzyme-linked probe for detecting, identifying or quantitating the presence of *Dekkera/Brettanomyces* yeast in a sample of interest, wherein the probe comprises a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

11. (Restated) The probe of claim 10, wherein the probing nucleobase sequence is selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

C2

12. (Amended) The probe of claim 10, wherein the probe is a peptide nucleic acid.

C3

16. (Amended) The probe of claim 10, wherein the probe is labeled with soy-bean peroxidase.

C4

18. (Amended) The probe of claim 10, wherein the probe is support bound.

19. (Restated) The probe of claim 18, wherein the probe exists attached to an array of probes.

C5

21. (Amended) A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample of interest, wherein one or more of the probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

C6

23. (Amended) The probe set of claim 21, wherein the probe set is specific for both the detection of *Dekkera/Brettanomyces* yeast as well as other organisms of interest in the same sample.

24. (Restated) The probe set of claim 23, wherein the probes of the set are independently detectable.

C7

25. (Amended) The probe set of claim 21, wherein some of the probes of the set are blocking probes.

26. (Amended) The probe set of claim 21, wherein all probes of the set are peptide nucleic acids.

C8

29. (Amended) The probe set of claim 21, wherein the probes are labeled with the enzyme soy-bean peroxidase.

C9

32. (Amended) The probe set of claim 21, wherein the probes are support bound.

C10

34. (Amended) A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera bruxellensis* yeast in a sample of interest, wherein the two or more probes specific for *Dekkera bruxellensis* yeast comprise a probing nucleobase sequence wherein at least portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5) and CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6) and sequences fully complementary thereto and of the same length.

46. (Restated) A method for detecting, identifying or enumerating yeast in a sample of interest, said method comprising:

a) contacting one or more species of yeast in the sample with one or more yeast specific enzyme-linked probes, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast; and

b) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample.

47. (Restated) The method of claim 46 further comprising the step of:

c) isolating the yeast using a filter as an isolation medium.

48. (Restated) The method of claim 47, further comprising the step of:

d) growing the isolated yeast by culture in media.

49. (Restated) The method of claim 48, wherein the culture is grown directly on the filter, under suitable culture conditions, by placing the filter in contact with media.

C11

61. (Amended) A method for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample; said method comprising:

a) contacting one or more species of yeast in the sample with one or more *Dekkera/Brettanomyces* yeast specific probes, under suitable hybridization conditions, to thereby form a probe/target sequence hybrid; and

b) detecting the presence, absence or amount of probe/target sequence hybrid and correlating the result with the presence, absence or number of *Dekkera/Brettanomyces* yeast in the sample;

wherein one or more of the *Dekkera/Brettanomyces* yeast specific probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

62. (Restated) The method of claim 61, wherein the probing nucleobase sequences of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

80. (Amended) A kit for performing an *in-situ* assay that detects, identifies or enumerates *Dekkera/Brettanomyces* yeast in a sample, wherein said kit comprises:

a) a filter for isolating yeast from a sample of interest;
b) culture media for growing the isolated yeast;
c) fixation solution for fixing grown yeast;
d) hybridization solution for imposing suitable *in-situ* hybridization conditions;
e) an enzyme labeled probe specific for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in the sample; and
f) one or more wash solutions for removing undesirable components after performing one or more steps of the assay.

81. (Restated) The kit of claim 80, wherein the fixation solution and the hybridization solution are the same solution.

82. (Restated) The kit of claim 80, wherein the soy bean peroxidase labeled probe is a peptide nucleic acid.

83. (Restated) A method for quantitating slow growing yeast in a liquid sample in less than 48 hours; said method comprising:

a) filtering a fixed volume of liquid using a filter having a pore size that does not allow the yeast to pass;

b) incubating the filter containing the yeast, in media and under culture conditions, for 45 or fewer hours to thereby grow microcolonies of yeast;

c) fixing the microcolonies of yeast to the filter;

d) contacting the microcolonies of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast;

e) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample; and

f) determining the quantity of yeast in the sample.

84. (Restated) The method of claim 83, wherein fixing the microcolonies of yeast to the filter and contacting the microcolonies of yeast with a yeast specific enzyme-linked probe are performed simultaneously using a single solution.

85. (Restated) The method of claim 83, wherein the number of CFU in the sample is determined.

C13

86. (Amended) The probe set of claim 10, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

87. (Amended) The probe set of claim 21, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.